

# Brie (mouse) and Brunello (human) reference libraries / Doench et al.

## SpCas9 KO-libraries

These reference libraries comes in a human a mouse version, with four suggested spacers for each gene. A scoring system (Rule Set 2 score) is applied and can be used to select a smaller number of spacers per gene, if of interest.

The spacers were selected for both low off-target activity, and high on-target activity.

The Brunello Library contains 1000 non-targeting controls that can be extracted by writing “Non-Targeting Control” in the Green Listed input box. The non-targeting controls have been given an arbitrary score from 1-1000, allowing you to specify how many non-targeting controls to include, by inserting the number in the Ranking option next to “limit to top”.

→ E.g. if you like to extract 25 non-targeting constructs from the Brunello Library. Choose Brunello. Input “Non-Targeting Control” in the input box. In the options box click “Ranking” and click the box next to “limit to top”. Input 25 in the box. Add adaptor sequences under the “Sequence modification” tab relevant for your cloning strategy. Click Run and extract the data.

More information about the libraries, as well as pooled plasmids can be accessed through Addgene:

<https://www.addgene.org/pooled-library/broadgpp-human-knockout-brunello/>

<https://www.addgene.org/pooled-library/broadgpp-mouse-knockout-brie/>

## Reference

Doench JG\*, Fusi N\*, Sullender M, Hegde M, Vaimberg EW, Donovan KF, Smith I, Tothova Z, Wilen C, Orchard R, Virgin HW, Listgarten J\*, Root DE\*. (Corresponding authors \*)

Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9.

Nat Biotechnol. 2016 Feb;34(2):184-91. doi: 10.1038/nbt.3437. Epub 2016 Jan 18. [link](#)